10

15

20

25

30

35

We claim:

- An isolated polynucleotide derived from a fungal source, which polynucleotide comprises a nucleotide sequence encoding an enzyme having β-glucosidase activity.
- 5 2. An isolated polynucleotide selected from the group consisting of:
 - (a) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL4 polypeptide having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
 - (b) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL4 polypeptide having at least 90% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
 - (c) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL4 polypeptide having at least 95% sequence identity to the amino acid sequence presented in Figure 2;
 - (d) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL4 polypeptide having the amino acid sequence presented in Figure 2;
 - (e) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL4 polypeptide having at least 95% sequence identity to the amino acid sequence presented as SEQ ID NO:2;
 - (f) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a BGL4 polypeptide having the amino acid sequence presented as SEQ ID NO:2;
 - (g) a nucleic acid sequence presented as SEQ ID NO:3, or the complement thereof; and
 - (h) a nucleic acid sequence that hybridizes, under high stringency conditions to the sequence presented as SEQ ID NO:3, or the complement or a fragment thereof, wherein said isolated polynucleotide encodes a polypeptide having the biological activity of a β-glucosidase
 - 3. The isolated polynucleotide of Claim 2, wherein % identity is calculated using the CLUSTAL-W program in MacVector version 6.5, operated with default parameters, including an open gap penalty of 10.0, an extended gap penalty of 0.1, and a BLOSUM 30 similarity matrix.

- 5. The isolated polynucleotide of Claim 2, wherein said polynucleotide is an RNA molecule.
- 6. The isolated polynucleotide encoding an enzyme having β -glucosidase activity, wherein the enzyme is derived from a Trichoderma source.
- 7. The isolated polynucleotide of Claim 6, wherein the enzyme is derived from Trichoderma reesei.
 - 8. An expression construct including a polynucleotide sequence (i) having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2), or (ii) being capable of hybridizing to a probe derived from the nucleotide sequence disclosed in Figure 2 under conditions of intermediate to high stringency, or (iii) being complementary to a nucleotide sequence having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2).
 - 9. A vector including the expression construct of Claim 8.
 - 10. A vector comprising an isolated polynucleotide of Claim 2, operably linked to control sequences recognized by a host cell transformed with the vector.
 - 11. A host cell transformed with the vector of Claim 9.,
 - 12. A host cell transformed with the vector of Claim 10.
 - 13. The host cell of Claim 12, which is a prokaryotic cell.
 - 14. The host cell of Claim 12, which is a eukaryotic cell.
 - 15. A recombinant host cell comprising a polynucleotide of Claim 2.
 - 16. The recombinant host cell of Claim 15, which is a prokaryotic cell.
 - 17. The recombinant host cell of Claim 15, which is a eukaryotic cell.
 - 18 A substantially purified BGL4 polypeptide with the biological activity of a β -gluogsidase, comprising a sequence selected from the group consisting of:
 - (a) an amino acid sequence having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
 - (b) an amino acid sequence having at least 90% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
 - (c) an amino acid sequence having at least 95% sequence identity to the amino acid sequence presented in Figure 2;
 - (d) an amino acid sequence presented in Figure 2;

RAZZOOD.

5

15

20

30

25

35

10

20

25





(e) an amino acid sequence having at least 95% sequence identity to the amino acid sequence presented as SEQ ID NO:2;

- (f) an amino acid sequence presented as SEQ ID NO:2;
- (g) a substantially purified biologically active fragment of the amino acid sequence presented as SEQ ID NO:2.
- 19. A method of producing an enzyme having β -glucosidase activity, comprising:
 - (a) stably transforming a host cell with an expression vector comprising a polynucleotide as defined in Claim2;
 - (b) cultivating said transformed host cell under condition suitable for said host cell to produce said β-glucosidase; and
 - (c) recovering said β -glucosidase.
- 20. The method of Claim 19 wherein the host cell is a filamentous fungi or yeast cell.
- 21. A purified enzyme having β -glucosidase activity prepared by the method of Claim 19—
- 22. A recombinant host cell comprising a deletion or insertion or other alteration in the bgl4 gene which inactivates the gene and prevents BGL4 polypeptide production.
 - 23. An antisense oligonucleotide complementary to a messenger RNA that encodes a BGL4 polypeptide having the sequence presented as SEQ ID NO:2, wherein upon exposure to a β -glucosidase-producing host cell, said oligonucleotide decreases or inhibits the production of β -glucosidase by said host cell.
 - 24. The antisense oligonucleotide of Claim 23, wherein the host cell is a filamentous fungi.
 - 25. A detergent composition, said composition comprising a polypeptide selected from the group consisting of:
 - (a) an amino acid sequence having at least 85% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
 - (b) an amino acid sequence having at least 90% sequence identity to the amino acid sequence presented in Figure 2 (SEQ ID NO:2);
 - (c) an amino acid sequence having at least 95% sequence identity to the amino acid sequence presented in Figure 2;
 - (d) an amino acid sequence presented in Figure 2;
 - (e) an amino acid sequence having at least 95% sequence identity to the amino acid sequence presented as SEQ ID NO:2;
 - (f) an amino acid sequence presented as SEQ ID NO:2;
 - (g) a substantially purified biologically active fragment of the amino acid sequence presented as SEQ ID NO:2.

30

35

5

15

20

25

30

35

- 26. A method of expressing a heterologous polypeptide having β -glucosidase activity in an Aspergillus species, comprising:
 - (a) Providing a host Aspergillus with an expression vector comprising a
 polynucleotide encoding a signal sequence linked to a polynucleotide
 encoding a heterologous β-glucosidase, thereby encoding a chimeric
 polypeptide;
 - (b) Cultivating said host Aspergillus under conditions suitable for said Aspergillus to produce said chimeric polypeptide, wherein said chimeric polypeptide is produced.
- 10 27. A method of producing ethanol, said method comprising the steps of:
 - contacting a biomass composition with an enzymatic composition comprising β-glucosidase 4 to yield a sugar solution;
 - b) adding to the sugar solution a fermentative microorganism; and
 - c) culturing the fermentative microorganism under conditions sufficient to produce ethanol,

wherein the biomass composition may be optionally pretreated.

- 28. The method of claim 27 wherein step (a) further comprises the addition of at least one endoglucanase.
- 29. The method of claim 27 wherein step (a) further comprises the addition of at least one cellbiohydrolase.
- 30. The method of claim 28 wherein step (a) further comprises the addition of at least one cellbiohydrolase.
- 31. The method of claim 27 wherein the pretreatment is with a dilute acid.
- 32. A method of producing ethanol, said method comprising the steps of:
 - a) contacting a biomass composition with an enzymatic composition comprising a β -glucosidase 4 and a fermentative microorganism; and
 - b) culturing the fermentative microorganism under conditions sufficient to produce ethanol,

wherein the biomass composition may be optionally pretreated.

- 33. The method of claim 32 wherein step (a) further comprises the addition of at least one endoglucanase.
- 34. The method of claim 32 wherein step (a) further comprises the addition of at least one cellbiohydrolase.
 - 35. The method of claim 33 wherein step (a) further comprises the addition of at least one cellbiohydrolase.
- 36. The method of claim 32 wherein the pretreatment is with a dilute acid.